The Influence of the Conditions of the Pet Trade on the Commensal Gastrointestinal Flora of Wild-Caught Tokay Geckos (*Gekko gecko*)

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Abstract: This study investigates the potential of the pet trade, particularly the conditions under which wild animals are captured and transported, to influence the community composition of the gastrointestinal flora and the prevalence of pathogens shed by Tokay geckos (*Gekko gecko*). The commensal enteric composition of individually housed Tokay geckos was characterized; and in an effort to understand the effects of importation on commensal enteric composition, experimental manipulations of density were conducted to mimic stressful overcrowding conditions in the pet trade. A total of 189 lactose-positive *Enterobacteriaceae* isolates were cultured from fecal samples obtained from 110 imported geckos. The top three most frequently cultured genera were *Citrobacter* (n = 114), *Klebsiella* (n = 29), and *Enterobacter* (n = 15). *Citrobacter* was the most frequently cultured genus from all geckos. The prevalence of *Citrobacter* spp. increased significantly from 55% among all individuals to 75% from grouped geckos ($P = 0.011$). The prevalence of *Salmonella enterica* subspecies *arizonae*, a common reptile-associated zoonotic pathogen, was ≤1% in individually housed geckos compared to 12% cultured from animals living in groups ($P = 0.0004$). The second most common genus cultured, *Klebsiella*, decreased significantly from 20% among individually housed geckos to 4% from all the combined animals ($P = 0.007$). The increase in prevalence of *S. enterica* subspecies *arizonae* and opportunistic pathogens like *Citrobacter* spp. illustrates that regulations aimed at decreasing overcrowding on imported reptiles might help to minimize pathogen shedding and transmission.

Key Words: *Enterobacteriaceae*, enteric flora, *Gekko gecko*, pet trade, reptile, Tokay gecko.

Introduction

The United States imported nearly 1.5 billion live animals between 2000 and 2006. Approximately 92% of those imports were designated for commercial purposes (the majority for the pet trade), of which, the third most abundant taxonomic group was reptiles (Smith *et al.*, 2009). Smith *et al.* (2009) concluded that little pathogen screening is implemented or required for imported animals, and 80% of shipments contained wild-caught animals. Of these live shipments, 69% of imports into the United States originated in Southeast Asia, a known region for emerging infectious diseases (Jones *et al.*, 2008; Smith *et al.*, 2009). Of particular significance, the ongoing multistate, multinational human salmonellosis outbreaks linked to reptiles illustrate the importance of the potential for pathogen transmission, such as *Salmonella*, by imported reptiles that are destined for the pet trade (Mermin and Hoar, 1997; CDC, 2003; Schröter *et al.*, 2004; Angulo *et al.*, 2010; Shin *et al.*, 2012). The Tokay gecko (*Gekko gecko*) is a reptile that is commonly imported for the pet trade and is native to Southeast Asia. The exact number of Tokay geckos imported into the United States annually is difficult to determine, but it is estimated to be in the millions (Schlaepfer *et al.*, 2005; Smith *et al.*, 2009). The Tokay gecko is a great model for understanding the potential for the movement of pathogens by a reptile through the international pet trade because it is a common pet reptile frequently handled by distributors and owners, is imported in large numbers, is wild caught, and originates from Southeast Asia (Cavendish, 2001; Schlaepfer *et al.*, 2005).

There have been no comprehensive studies describing the normal gastrointestinal flora of reptiles (Mathewson, 1979; Cooper *et al.*, 1985; Jacobson, 2007). However, reports describing *Salmonella* spp. from reptiles and their association with disease in humans are numerous (Mermin *et al.*, 2004; Aiken *et al.*, 2010; Fookes *et al.*, 2011). *Salmonella* spp. can be a pathogen in reptiles but are generally considered to be commensal organisms of reptiles (Jackson and Jackson, 1971; MacNeill and Dorward, 1986; Gopee

et al., 2000; Nakadai *et al.*, 2005; Jacobson, 2007; Hatt *et al.*, 2009) compared to humans where it is a significant pathogen. This information is based on studies primarily conducted on captive reptiles. Reports of *Salmonella* spp. prevalence in their wild counterparts varies widely, from an absence reported in some studies to 12–100% depending on the species (Briones *et al.*, 2004; Richards *et al.*, 2004; Saelinger *et al.*, 2006; Hidalgo-Vila *et al.*, 2007; Hoelzer *et al.*, 2011). *Salmonella enterica* shed from reptiles remains a major public health concern because it is estimated that reptile-associated salmonellosis accounts for 74,000 cases of salmonellosis annually in the United States (CDC, 2003; Mermin *et al.*, 2004). *Salmonella* spp. along with other enteric organisms belong to a diverse family known as *Enterobacteriaceae*, of which several members are considered important pathogens, and others are commonly associated with commensal gastrointestinal flora. Specifically, lactose-fermenting *Enterobacteriaceae* such as *Escherichia* spp., *Citrobacter* spp., *Klebsiella* spp., *Serratia* spp., and *Enterobacter* spp. (Guentzel, 1996; Janda and Abbott, 2006; Todar, 2008) are typically nonpathogenic but have the potential to produce opportunistic infections (Guentzel, 1996). Describing the composition of commensal flora shed by reptiles is important also because these bacteria are known to cause opportunistic infections in high-risk groups, such as children, the elderly, and immunocompromised individuals, who may be handling these animals or their environments (Gerba *et al.*, 1996; Kendall *et al.*, 2003; Yang *et al.*, 2003; Whitley and Monto, 2006).

The significance of the intestinal flora was first explored in the early 1900s (Metchnikoff, 1908; Douglas, 1911) but recently has revolutionized our understanding of the role of normal flora in both human and animal health (Neish, 2002; Dethlefsen *et al.*, 2006; Guarner, 2006). The normal intestinal flora of an organism is influenced by several factors including diet, age, sexual reproduction, environmental conditions, and stress (O'Hara and Shanahan, 2006). Thus far, studies on the effects of stress associated with crowding and its relationship to the colonization and shedding of pathogens important to human health, like *Salmonella* spp., are limited to domestic animal production facilities and, sparsely, captive wildlife species (Richards *et al.*, 2004; De Passillé and Rushen, 2005; Hatt *et al.*, 2009; Ball *et al.*, 2011). Also, the pet trade, like the livestock industry, moves animals across regional and international borders, which harbor pathogens known to cause disease in people (Daszak *et al.*, 2000; Brown, 2004; Pavlin *et al.*, 2009). Specifically, the commensal flora and the potential movement of enteric pathogens by domestic animals across international borders has been investigated, but little information exists regarding either the commensal enteric flora harbored by wildlife commonly transported for the pet trade or the effects of associated stress on the composition of the normal flora (Okeke and Edelman, 2001). The authors propose that, much like in production animals (Rostagno, 2009), shipping and other import conditions that cause stress could disrupt the normal flora of Tokay geckos and possibly promote the overgrowth and colonization of enteric bacteria known to cause disease in people.

The first objective of this study was to describe the culturable lactose-fermenting *Enterobacteriaceae* bacteria (from here forward: the enteric commensal flora) from the feces of Tokay geckos. To the authors' knowledge, this is the first report to describe the enteric flora of wild-caught Tokay geckos imported for the pet trade. The second objective was to describe the effects that overcrowding had on the diversity and prevalence of these flora, including the rate of shedding of lactose-positive *S. enterica* spp. The authors specifically hypothesized that overcrowding creates conditions that have the potential to alter the normal flora composition, resulting in a decreased diversity of enteric bacterial isolates and an increase in *S. enterica* spp. prevalence.

Materials and Methods

Geckos were captured by a reptile distributor following standard business practices from rural locations outside Jakarta on the island of Java and shipped via airfreight to the University of Georgia's (UGA) College of Veterinary Medicine (Athens, GA). Geckos were hand captured from homes, barns, and other anthropogenic structures and shipped within 5 days of capture (range 1–5 days). Immediately after capture, geckos were housed individually in clean containers for shipping to minimize environmental bacterial contamination. These containers were not opened until their arrival at UGA. One hundred fifty geckos, a mix of males and females, were shipped to UGA in two batches, 60 in March 2009 (Batch 1) and 90 in July 2009 (Batch 2). A total of 50 geckos from Batch 1 and 60 geckos from Batch 2 were used for this study. Their husbandry requirements were met as previously described (Bartlett and Bartlett, 1995). Briefly, they were fed a standard diet of crickets and mealworms. The temperature and humidity were maintained at 26.6°C ± 4° (79.8°F ± 8°) and 50–70%, respectively, and the lighting, provided by full-spectrum lights, was on a 12 h cycle. The geckos were monitored twice daily. At arrival, the first batch of animals was housed individually in clear propylene boxes for 10 days, during which time fecal samples were collected from each animal. Once fecal samples were collected, individual geckos were randomly assigned to three groups—low density (n = 5), medium density (n = 15), and high density (n = 30)—and then transferred to their respective enclosures measuring 61 cm wide × 91 cm deep × 183 cm high. The second batch of individually housed geckos was imported in July. As before, fecal samples were collected from animals while they were housed individually, after which 15 geckos from the second batch were assigned to each of the pre-existing density groups (low, medium, and high), and a fourth temporal group was created (n = 15). The temporal group was comprised of only geckos from the second batch and was created to determine whether there was any difference in genera shed from the second compared to the first batch of imported geckos. All groups were housed in the same size enclosures and under the same environmental conditions and feeding regimes. The only difference among the groups was density of the animals in each enclosure. In September, individuals were removed from enclosures and placed in separate propylene containers until they defecated. All animal handling and care was approved by the University of Georgia's Institutional Animal Use Care Committee (A2008, 12-051).

Fecal samples were embedded in sterile containers with 1 mL of sterile water to maintain moisture until submission to the laboratory. Fecal samples were submitted and processed within 12 h of collection. A 10 μL loop was used to

Table 1. The prevalence of isolates to the species level among individuals and combined geckos.

Bacteria	Individual	Combined
Citrobacter freundii	57	30
Citrobacter braakii	1	0
Citrobacter koseri	4	3
Citrobacter youngae	6	6
Citrobacter sp.[a]	7	0
E. coli	7	0
Enterobacter aerogenes	9	0
Enterobacter amnigenus biogroup 2*	0	1
Enterobacter sp.[b]	4	1
Klebsiella oxytoca	16	0
Klebsiella pneumoniae	11	2
Kluyvera sp.[c]	9	2
Pantoea sp.[d]	1	1
Salmonella arizonae	1	6
Serratia odorifera biogroup 1*	1	0
Serratia sp.[e]	3	0
Total	137	52

For isolates *Citrobacter* sp.[a], *Enterobacter* sp.[b], *Kluyvera* sp.[c], *Pantoea* sp.[d], and *Serratia* sp.[e], the species level was unable to be determined the Analytical Profile Index (API20E, Biomerieux, Durham, NC). For the isolates indicated with an asterisks (*), the number following name represents the biogroup/biovar that isolate belongs to and was identified by the Analytical Profile Index (API20E, Biomerieux, Durham, NC).

streak the diluted fecal solution on MacConkey media plates, and the plates were incubated at 37°C ± 2° (98.6°F ± 2°) for 24 h. One sample of each morphologically distinct group of colonies was collected and re-streaked separately back onto MacConkey. If the colonies were later identified as the same genus and species, only one of the isolates was used. Pure isolates were suspended in freezer media consisting of 1% peptone and 15% glycerol and frozen at −80°C (−112°F) until further analysis. Standard methods were used to determine the identity of isolated organisms, including plating on a selective and differential media and performing standard biochemical tests as recommended in the Manual of Clinical Microbiology (Murray *et al.*, 2003). If there were conflicting biochemical test results, rapid Analytical Profile Index strips (API20E, Biomerieux, Durham, NC) were used to definitively determine the identification of the isolate. Isolates were identified to the species level, except for a few cases where the Analytical Profile Index was unable to provide a conclusive result. Statistical comparisons were made at the genus level.

Table 2. Prevalence of bacterial genera cultured from individually housed Tokay geckos imported from Indonesia in 2 transport batches.

Genera	Batch 1 (%)	Batch 2 (%)
Citrobacter spp.	59	52
Klebsiella spp.	20	19
Enterobacter spp.	7	11
Kluyvera sp.	7	6
Echerichia coli	0	8
Serratia sp.	2	4
Pantoea sp.	2	0
S. enterica subspecies *arizonae*	2	0
Total number of isolates[a]	54	83

[a] Values are total number of isolates cultured.

Statistical analyses were performed using SAS V 9.2 (SAS Institute Inc., Cary, NC). An analysis of covariance (ANCOVA) was used to compare diversity (total number of genera) between bacteria cultured from individually housed geckos and all geckos combined. Housing density (low, medium, and high), number of animals, and number of isolates were included as covariates. Also, a chi-square analysis was used to compare the prevalence of each genus among all the individuals to all the combined animals irrespective of density level. A chi-square analysis was used to compare the prevalence of each genus between individual and combined groups for each housing density level. All hypothesis tests were 2-sided, and the significance level was set at $\alpha = 0.05$.

Results

Overall, we recovered 189 lactose-positive *Enterobacteriaceae* isolates from 110 geckos (Table 1). The most frequently cultured genera were *Citrobacter* (n = 114), *Klebsiella* (n = 29), *Enterobacter* (n = 15), and *Kluyvera* (n = 11). Seven isolates of each *S. enterica* subspecies *arizonae* and *Escherichia coli* were cultured. *Serratia* (n = 4) and *Pantoea* (n = 2) made up the least commonly cultured genera.

For the 110 individually housed geckos, eight genera of lactose-positive *Enterobacteriaceae* were isolated (Table 1). The pattern of diversity was such that *Citrobacter*, *Klebsiella*, and *Enterobacter* were the most prevalent genera; *Kluyvera*, *E. coli*, and *Serratia* were the next most common genera cultured; and *Pantoea* and *S. enterica* subspecies *arizonae* made up <1% of the isolates cultured. There was little difference in the diversity (number of genera) of enteric bacteria between the two batches of imported animals (Table 2).

There was no statistical significance in the difference of diversity of genera recovered between individually housed geckos and all combined geckos ($P = 0.7$) or in the comparison of the different density groups ($P = 0.06$), number of animals ($P = 0.6$), or number of isolates ($P = 0.8$). However, there were some notable differences in the prevalence

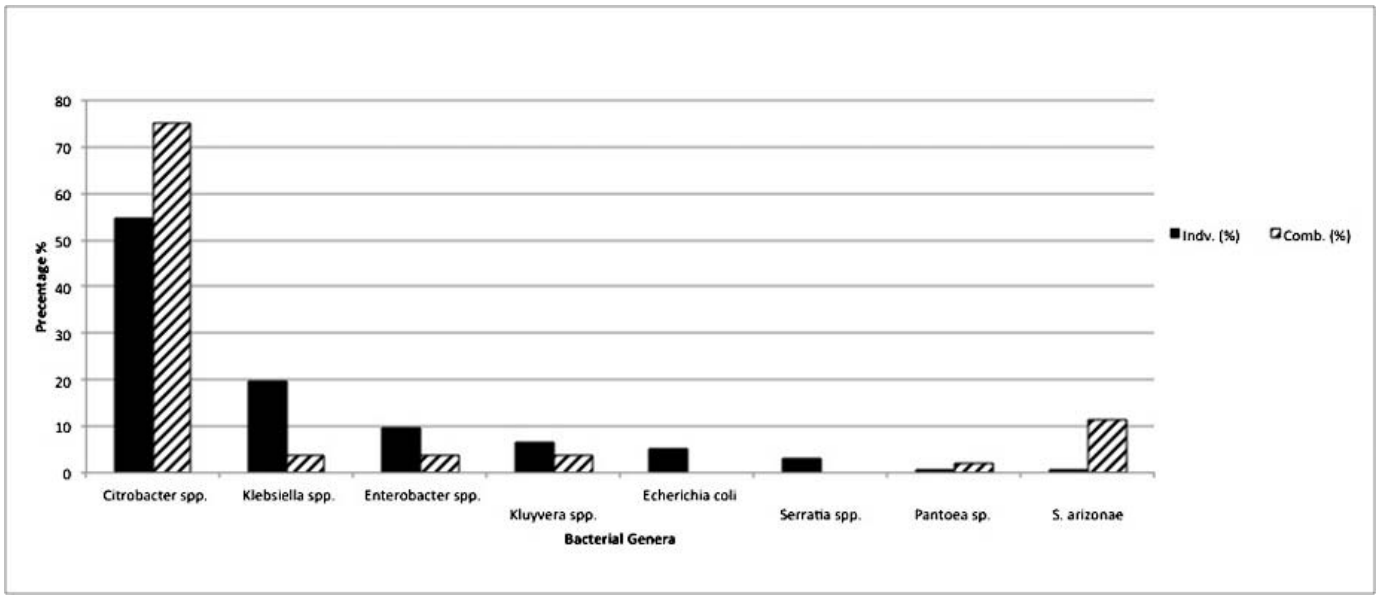

Figure 1. Prevalence of genera from all the individually housed geckos was compared to geckos living in combined groups. After animals were combined into groups, 76 animals survived to the end of the study, and of those, 52 lactose-positive isolates were isolated from 44 geckos.

of genera from individually housed geckos compared to the combined geckos. *Citrobacter* was the most frequent genus cultured from all geckos, and its prevalence increased from 55% among all individuals to 75% from grouped geckos ($P = 0.011$; Fig. 1). The prevalence of *Klebsiella* spp. cultured from all the individually housed geckos decreased from 20% to 4% from all the combined animals ($P = 0.007$; Fig. 1). The difference in prevalence of *Enterobacter* and *Kluyvera*, the two other most frequently cultured genera, was not significant ($P = 0.2$; $P = 0.47$, respectively). *Salmonella enterica* subspecies *arizonae* was cultured from three of the four combined density groups compared to a single individually housed gecko. Thus, the prevalence of *S. enterica* subspecies *arizonae* cultured from all the combined animals rose from 1% to 12% ($P = 0.0004$).

The prevalence of all genera was compared between the individually housed geckos assigned to a specific density and once geckos were combined in that group. A statistically significant increase was observed in the prevalence of *Citrobacter* cultured from the low-density group, 36 to 82% ($P = 0.014$; Fig. 2b). Additionally, the prevalence of *S. enterica* subspecies *arizonae* increased in high density from 0 to 19% ($P = 0.0005$; Fig. 2d). Interestingly, the prevalence of *Enterobacter* decreased from 31 to 0% in the temporal group ($P = 0.033$; Fig. 2a). All other comparisons of prevalence between the individually housed geckos assigned to a specific density and once combined in that group by genus were not statistically significant.

Discussion

Previous studies have demonstrated a positive relationship between diverse and stable gastrointestinal microbiota and the prevention of pathogen colonization (Levy, 2000; Guarner and Malagelada, 2003). Stress, other host physiologic changes, or the use of antibiotics can result in disruptions of microbial communities and can lead to either pathogen colonization or overgrowth of resident bacteria (Katouli *et al.*, 1994). Our results support the first part of our hypothesis, that housing geckos together influences the composition of their enteric flora. Housing geckos together is presumably stressful because of their natural history as a solitary, territorial, and aggressive lizard (Holdeman *et al.*, 1976; Lizko, 1987; Bailey and Coe, 1999; Knowles *et al.*, 2008). In addition to their predisposition to stress attributable to their natural history, captivity, frequent exposure to people, and handling have long been documented as stressors in production animals, wildlife, and reptiles (Guillette Jr *et al.*, 1995; Grandin, 1997; Moore *et al.*, 2000; Dickens *et al.*, 2010). A positive correlation between stress and changes in the microbial flora has been documented by several studies involving production animals and wild-caught animals (Berends *et al.*, 1996; Smith *et al.*, 2002; Bach *et al.*, 2004; Burkholder *et al.*, 2008; Rostagno, 2009; Godoy and Matushima, 2010). Although we did not use a quantitative method to measure stress in these animals, and cannot definitively associate this change in microbial flora with stress, there is a substantial amount of literature that supports our theory that these geckos were indeed stressed.

The commensal enteric flora (the isolates that were cultured from geckos immediately upon arrival from Indonesia) was comprised of genera previously described from reptiles (Mader, 2005). Although we expected to find *S. enterica* subspecies *arizonae*, *Citrobacter*, *Enterobacter*, *Klebsiella*, and *E. coli* based on previous studies of other reptiles, comparisons with this study are difficult because of differences associated with laboratory methodologies, different host species, their life stage, diet, and other factors and, most important, because most previous studies describe clinically diseased animals (Mathewson, 1979; Cooper *et al.*, 1985; Graves *et al.*, 1988; Gordon and Cowling, 2003; Mahajan *et al.*, 2003; Mader, 2005; Brown *et al.*, 2007; Jacobson, 2007; Johnson *et al.*, 2008; Martin *et al.*, 2010). Few reports describe the commensal enteric flora of geckos

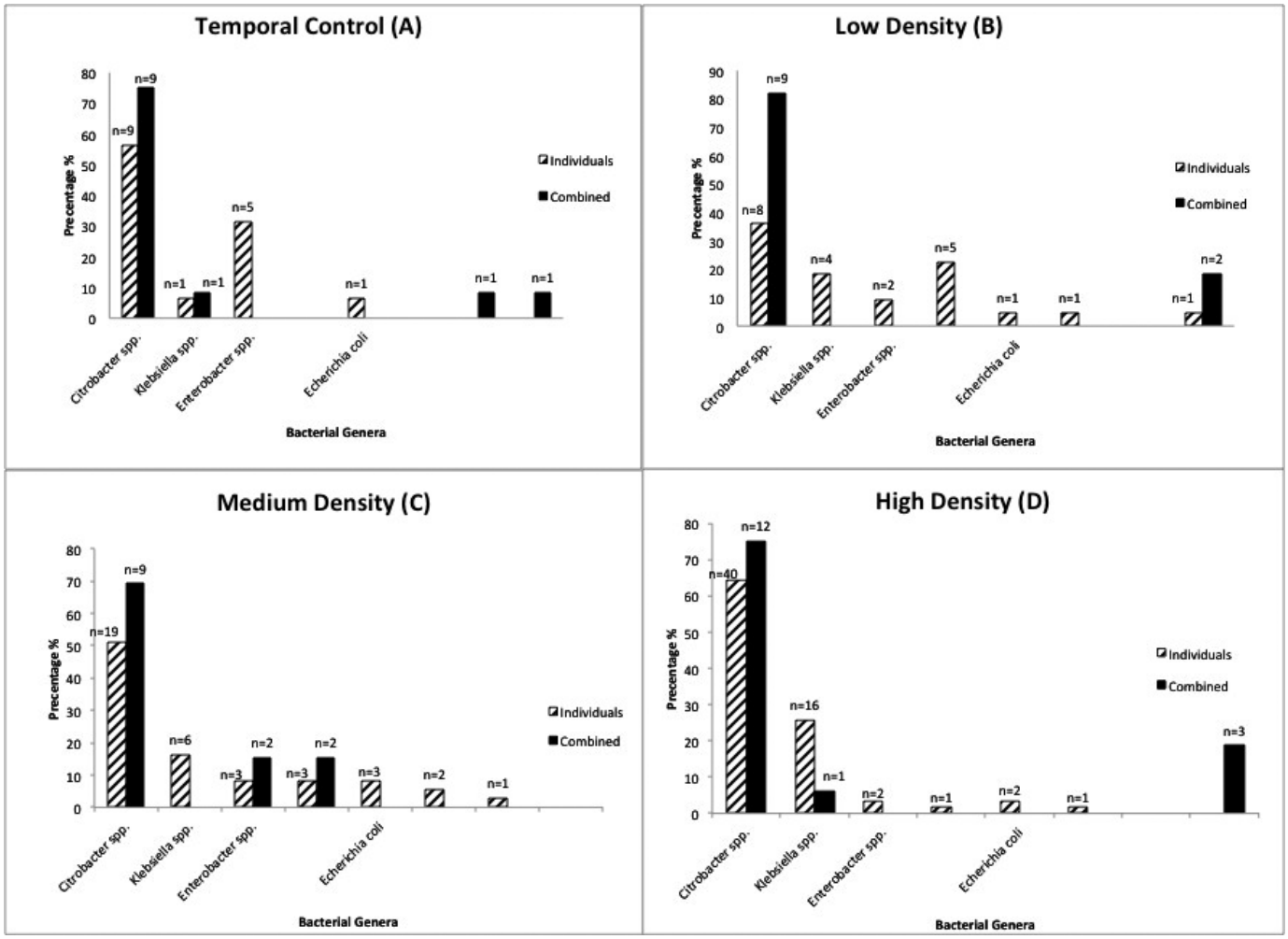

Figure 2. The prevalence of genera cultured from all individually housed Tokay geckos imported from Indonesia and then assigned to a specific density group was compared to their respective combined density group: (a) represents the genera cultured from the individuals assigned to the temporal group (n = 15) and the isolates cultured from the animals in the combined temporal group (n = 15); (b) represents the genera cultured from the individuals assigned to the low-density group (n = 20) and the isolates cultured from the combined low-density group (n = 15); (c) represents the genera cultured from the individuals assigned to the medium-density group (n = 30) and isolates from the animals in the combined medium-density group (n = 17); and (d) represents the prevalence of genera recovered from the individuals assigned to the high-density group (n = 45) and the isolates cultured from the animals in combined high-density group (n = 29). The decrease in the number of animals belonging to the combined groups is the result of mortality.

(Graves *et al.*, 1988), because the majority focus on the prevalence of *Salmonella* spp. (Hoff and White, 1977; Mathewson, 1979; Cooper *et al.*, 1985; Murphy and Myers, 1993; Callaway *et al.*, 2011). This is the first report that studied both the composition of culturable lactose-fermenter enteric commensals and *Salmonella* spp. in wild-caught Tokay geckos under the stressful conditions associated with the pet trade.

The two batches of geckos imported for the study were originally collected from two different areas of Java; however, their enteric composition was similar. In addition to being similar to previous reports, the similarity among batches supports our notion that individual composition data are a good representation of the normal flora of the Tokay gecko. *Salmonella enterica* subspecies *arizonae*, *Citrobacter*, *Enterobacter*, and *Klebsiell*a have all been commonly reported in association with the gastrointestinal flora of reptiles (Mathewson, 1979; Graves *et al.*, 1988; Richards *et al.*, 2004; Ebani and Fratini, 2005; Janda and Abbott, 2006; Jacobson, 2007). In addition, all have been reported to cause disease in both reptiles and humans (Ebani and Fratini, 2005; Jacobson, 2007).

The authors' second hypothesis was that overcrowding may promote the shedding of pathogenic bacteria, a phenomenon that has been shown specifically with the shedding of *S. enterica* in livestock (De Passillé and Rushen, 2005; Ball *et al.*, 2011). The data illustrated a significant increase in the prevalence of *S. enterica* subspecies *arizonae* cultured from the individual geckos (<1%) compared to the animals in the combined group (12%). Also, an increase in density facilitates transmission of this pathogen, and these two mechanisms are difficult to separate. The authors' did observe a statistically significant increase in the prevalence of *S. enterica* subspecies *arizonae* cultured from all the

individually housed geckos as compared to all of the geckos combined in groups. This is consistent with a separate but related study that described the prevalence of non–lactose-fermenting *Salmonella* spp. from these geckos (Smith *et al.*, 2012). However, the authors did not observe an increase in the prevalence of *S. enterica* subspecies *arizonae* among the different density groups as seen in Smith *et al*. (2012). A possible explanation for the increase in the overall prevalence, but not an increase in prevalence between the different density treatment groups, could be that stress from simply cohabitating was enough to induce a change in the normal flora and promote *S. enterica* subspecies *arizonae* shedding, regardless of final density. Therefore, this would make any additional stress from the increase in density irrelevant because the geckos were already sufficiently stressed. If the overcrowding does result in a higher prevalence of pathogen shedding, like in livestock, then it is prudent to apply management modifications during shipping and handling to reduce stress to diminish the potential public health risk.

The authors observed a decrease in the diversity of lactose-fermenting enteric flora. In other animal models, the disruption of normal flora attributable to stress results in decreased bacterial diversity, which promotes pathogen colonization. For example, a study in mice demonstrated that food deprivation and harassment destabilized their enteric microbial communities, diminishing their enteric diversity and increasing their susceptibility to colonization by *Citrobacter rodentium*, an enteric pathogen (Bailey *et al.*, 2010). Thus, maintaining a diverse healthy gastrointestinal microbial community is important because it protects against pathogen colonization (Lu and Walker, 2001; Dillon *et al.*, 2005; Dowd *et al.*, 2008; Chinnadurai and DeVoe, 2009). Although it is unclear whether stress resulted in a decrease in lactose-fermenting enteric flora diversity, it would be prudent to promote conditions that do not decrease gastrointestinal microbial diversity in pet reptiles that are known reservoirs of human pathogens because of the potential to increase the prevalence of pathogen colonization and, ultimately, shedding. The authors are assuming stress is, in part, responsible for the significant increase in prevalence of both *Citrobacter* and *S. enterica* subspecies *arizonae* and changes in the diversity. However, these changes could also be attributed to an inherent trait of the particular bacteria that allows it to outcompete other resident and transient flora for resources or other host factors (Ehrlich *et al.*, 2008; Lutgendorff *et al.*, 2008; Sekirov *et al.*, 2010).

Interestingly, although there was an increase in the prevalence of *Citrobacter* and *S. enterica* subspecies *arizonae*, there was also a significant decrease in *Klebsiella* cultured from the individuals (second most common genus; 19.7%) compared with the combined animals (3.8%). The prevalence of the other two most commonly isolated genera (*Enterobacter* and *Kluyvera*) from the combined animals decreased compared to the individuals. Also, there was a complete loss of 2 genera (*Serratia* and *E. coli*) in the combined groups. However, the differences in the diversity of genera between individual and combined groups were not statistically significant. Thus, the authors' initial prediction that as the density of geckos was artificially increased the diversity of culturable lactose-fermenting enteric flora would decrease was not validated. However, there was a consistent decrease in diversity between individuals and combined groups across all groups except the temporal group, which was comprised of geckos from the second shipment. To illustrate this point, the third most frequently isolated genus from the individual groups, *Enterobacter*, was recovered from only 1 of 4 combined groups. The increase in prevalence of a few genera and the decrease of others is one explanation for the change in diversity observed. The lack of statistical significance could be a result of the inability of analyses to detect differences when the range in the numbers of genera was too narrow (e.g., from 7 in the individuals to 2 in the combined groups).

Interestingly, there was no decrease in the diversity of lactose-fermenting *Enterobacteriaceae* cultured between the individual temporal group and the respective combined group. This group was created to determine whether there was a difference in the genera shed from the second batch compared to the first. The lack of change in diversity could be attributable to the shorter period of time these animals spent in the combined group or because it was compromised solely of animals from the second batch. Because the combined temporal group spent the least amount of time together, and the diversity remained the same compared to the other combined density groups, it would appear the diversity of commensal enteric flora is influenced by the amount of time geckos are housed in groups. A limitation of this study was the lack of repeated sampling at different time points to demonstrate that diversity truly decreased and was not just normal fluctuation of the microbial community.

Changes in lactose-fermenting *Enterobacteriaceae* shed by reptiles are important to monitor because they could provide insight into how stressful conditions similar to those currently employed during importation, shipping, and handling of pets influence enteric commensal flora composition. Although every effort was made through rapid shipping and individual housing to minimize effects, the enteric flora described was isolated once geckos arrived at the University of Georgia and may have already been affected by factors such as transport-associated stress. Thus, the description of enteric flora in this study should be interpreted with caution, although they are consistent with previous studies of reptiles and provide a unique description of commensal enteric flora of Tokay geckos, specifically. The authors focused on the lactose-fermenting *Enterobacteriaceae* because several members of this family are primary pathogens and some are important opportunistic pathogens, but this family represents a fraction of the complete flora of these animals, and any interpretations are limited to a subset of the normal flora.

Regulations are currently in place to diminish stress and prevent pathogen shedding in production animals (Sofos, 2008; Adam and Brülisauer, 2010), but the pet trade, which is similar in many respects, lacks these regulations. Promoting humane conditions for the importation of pet reptiles is vital for welfare concerns but may also help to maintain intact diverse microbial communities that protect individual animals from pathogen colonization and prevent pathogen dissemination. Maintaining diverse normal flora communities has many benefits to health, and this research suggests that stocking density alters a subset of this flora, decreasing diversity of commensal enteric bacteria and promoting the shedding of *S. enterica* subspecies *arizonae*. The increased prevalence of *S. enterica* subspecies *arizonae* is an important

finding because the popularity of reptiles as pets continues to increase and will likely mean that reptile-associated salmonellosis will continue to be a significant public health concern (Sanyal *et al.*, 1997; CDC, 2003). Future studies should focus on teasing apart the complex interactions between the host and the microbial communities and how sex, reproductive status, diet, stress, and environment influence the composition of the flora. Also, it would be beneficial to track the changes in normal flora at the individual level. Finally, acquiring and culturing fecal samples prior to transport in Indonesia, which was logistically impossible during our study, to compare them to samples taken immediately upon arrival, would ultimately allow the best representation of true shipping conditions and a more accurate description of the normal flora prior to exposure to stressors.

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